GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. _____

Project No./(Center No.) G-33-T05 R5479-5A0 GTRC/~~GIT~~ DATE 2 / 16 / 87

Project Director: Dr. Leon H. Zalkow School/~~XXX~~ Chemistry

Sponsor: DHHS/PHS/NIH/National Cancer Institute

Agreement No.: Grant No. 5 R01 CA31490-05

Award Period: From 12/1/86 To 11/30/87 (Performance) 2/29/88 Reports

Sponsor Amount:	New With This Change	Total to Date
Contract Value: $		$ 197,935
Funded: $		$ 197,935

Cost Sharing No./(Center No.) _____ Cost Sharing: $ _____

Title: Semisynthetic Pyrrolizidine Alkaloid Antitumor Agents

ADMINISTRATIVE DATA — OCA Contact E. Faith Gleason X-4820

1) Sponsor Technical Contact:

J.A.R. Mead, PH.D.
Division of Cancer Treatment
National Cancer Institute
5333 Westbard Ave.
Bethesda, MD 20892
(301) 496-8783

2) Sponsor Issuing Office:

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Grant Management Officer
National Cancer Institute
National Institutes of Health
5333 Westbard Ave.
Bethesda, MD 20892

Military Security Classification: N/A
(or) Company/Industrial Proprietary: _____

ONR Resident Rep. is ACO: _____ Yes X No
Defense Priority Rating: _____

RESTRICTIONS

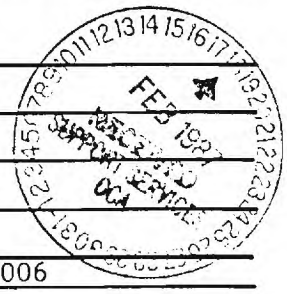
See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval — Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT. No equipment may be purchased within the last 6 months of the Final year of an NIH grant.

COMMENTS:

Continuation of G-33-T04



SPONSOR'S I.D. NO. 02.108.001.87.006

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GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

N-B SR-300

Date 12/30/87

Project No. G-33-T05 School/~~XXX~~ Chemistry

Includes Subproject No.(s) N/A

Project Director(s) L. H. Zalkow GTRC / ~~XXX~~

Sponsor DHHS/PHS/NIH/National Cancer Institute

Title Semisynthetic Pyrrolizidine Alkaloid Antitumor Agents

Effective Completion Date: 11/30/87 (Performance) 2/29/88 (Reports)

Grant/Contract Closeout Actions Remaining:

- [ ] None
- [X] Final Invoice or Final Fiscal Report
- [ ] Closing Documents
- [ ] Final Report of Inventions
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other

Continues Project No. G-33-T04 Continued by Project No. G-33-T06

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Angela DuBose
Russ Embry

FORM OCA 69.285

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER CA-31490-06	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Leon. H. Zalkow	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION Georgia Tech Research Corp.	Dec. 1, 1987	Nov. 30, 1988

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*

Semisynthetic Alkaloid Antitumor Agents

(SEE INSTRUCTIONS)

1. There have been no changes in our objectives, specific aims or experimental design and methods.

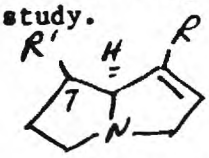
2. Studies conducted during current budget year:

Using the 3P31 in vivo antitumor assay at NIH, until its discontinuance, we continued to accumulate structure-activity information which hopefully will ultimately lead to an explanation of the mechanism of action of these compounds and to the rational design of the most effective antitumor agent. Following are listed the compounds synthesized and submitted for screening during this period, and comparison with our best drug to date, namely NSC 376359. NSC 612381 showed only marginal activity, suggesting that para electron releasing groups on the phenyl ring are detrimental. This is consistent with a mechanistic explanation involving alkylation at the C9 position by a biopolymer. NSC 610759 was found to be inactive, adding support to our in vitro evidence suggesting NSC 376359 is a DNA crosslinking agent, thus requiring two active functional groups (C7 & C9). NSC 610760 was toxic at 200 mg/Kg and inactive at lower doses. This is in contract to its excellent activity in the in vitro soft agar colony forming assay using A204 human rhabdomyosarcoma cells. We believe this suggests that too good a leaving group at C7 can lead to a lack of discrimination when the drug acts as an electrophile. No in vivo results are yet available on the bifunctional compounds NSC 614643 and 614644. All of the above mentioned compounds are N-oxides. Not unexpectedly, dihydroindicine N-oxide (NSC 600090), was found to be inactive, providing support for the assumption that the N-oxides are metabolically converted into the active pyrroles. Surprisingly, dihydroindicine (NSC 600089) was inactive but toxic at doses from 650 mg/Kg down to 162 mg/Kg (test reconfirmed), whereas the isomeric 7-(-)-trachelanthylplatynecine (NSC 610331) was both inactive and non-toxic). This strange toxicity of NSC 600089 remains unexplained (indicine itself is non-toxic at very high dose!). A number of other compounds have been synthesized and are at various stages of development for submission to the new NCI in vitro screen.

In the in vitro soft agar colony forming assay using A204 human rhabdomyosarcoma cells, NSC 614644 showed comparable activity to NSC 376359, suggesting it could be considered a prodrug. The corresponding free base EGK-24 was even more active, being considerably more active than the free base (EGK-25) of NSC 376359. The carbamate N-oxide NSC 614643, and its free EGK-20 were somewhat less active than NSC 614644. Several naturally occurring pyrrolizidine alkaloids were screened, but none were comparable to the semisynthetic compounds containing an aromatic ring in the side chain. We have developed a new and novel synthesis of supinidine from retronecine which will allow us to determine whether it is necessary to have a leaving group at C-7.

During this period we have spent considerable time developing the procedures for determining the partition coefficients of the drugs (N-oxides have not previously been measured), and their corresponding free bases, and in

measuring the kinetics of the alkylation reactions of the pyrroles, chemically derived from the drug N-oxides, with 4-p-nitrobenzylpyridine. These experimental procedures have been developed using considerable amounts of automation and data on a number of compounds have been obtained. The purpose of this investigation is to obtain physical-chemical information on the various drugs to correlate with the screening data in a structure-activity study.



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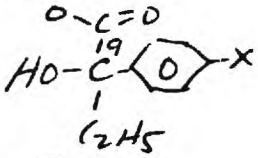

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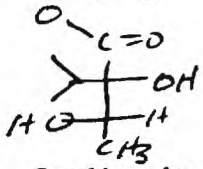

EGK-25	R = A, X = Cl, R' = OH
NSC-376359	N-oxide of EGK-25
NSC-612381	R = A, X = OCH_3, R' = OH, N-Oxide
NSC-610759	R = CH_3, R' = A, X = Cl, N-Oxide
NSC-610760	R = R' = A, X = Cl, N-Oxide
EKG-20	R = A, X = Cl, R' = $OCONHC_2H_5$
NSC-614643	N-Oxide of EGK-20
EGK-24	R = A, X = Cl, R' = $OCONHC_2H_5$
NSC-614644	N-Oxide of EGK-24
NSC-600090	R = B, R' = OH, N-Oxide, Dihydro
NSC-600089	R = B, R' = OH, Dehydro
NSC-610331	R = CH_2OH, R' = B, Dihydro

Studies have been conducted on DNA crosslinking and DNA strand breaks with the pyrrolizidine alkaloids (PAs) incubated with tumor cells using the DNA alkaline elution assay. A small number of DNA strand breaks were produced by indicine N-oxide. This could be due to formation of oxygen radicals or to other damage to the DNA. Single strand breaks have not been seen with other PAs. DNA crosslinking has been seen with the NSC-376359 and EGK-25, by the diester NSC-610760 and its free base and by indicine, but not indicine N-oxide. The PAs are not particularly strong crosslinking agents compared with other crosslinking agents and good dose-response curves are not seen. There appear to be two clasess of PAs, those that are moderate crosslinkers with considerable in vitro cytotoxicity, where the N-oxide is more active than the base, and non-crosslinkers where the base is more active than the N-oxide.

Based on our hypothesis that PA N-oxides are activated to pyrrolic alkylating intermediates by an iron catalyzed reaction, we have been looking at the effect of iron chelating agents on the cytotoxicity of PAs to tumor cells in culture. We have so far not seen an effect of the iron chelators on cytotoxicity. Other metal ions may, however, catalyze the oxidation of PAs.

We have continued our studies to develop an animal model for the hepatotoxicity of antitumor PAs. We have been unable to obtain consistent signs of hepatotoxicity with indicine N-oxide administered, even at toxic doses i.v. to dogs, rats or mice. When indicine N-oxide is administered p.o. to mice hepatic necrosis is seen. This may form the model for hepatic toxicity and work is continuing with other PAs administered p.o.

Publications: Structure of 2a-Bromo-1β,7β-epoxytrachelanthamidine: A New Heterocyclic Ring System. Glinski, J. A.; VanDerveer, D.; Zalkow, L. H. Acta Cryst. 1986, C42, 1239-1242.